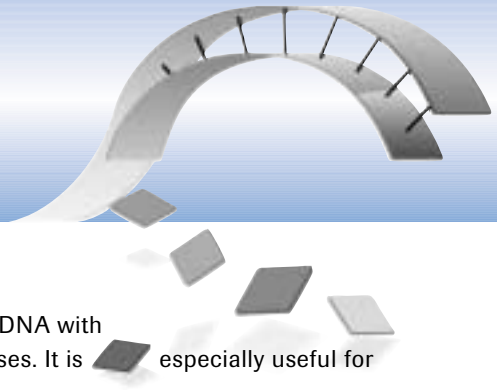
# *Pwo DNA Polymerase*

**Specification**

| Catalog No. | **1654 101**<br>**Thermostable DNA polymerase** |
|---|---|
| Origin | Pyrococcus woesei, recombinant in E. coli RM82 |
| Appearance | clear, colorless solution in Tris-HCl, 20 mmol/l;<br>KCl, 100 mmol/l; EDTA, 0.1 mmol/l; DTE, 1 mmol/l;<br>Nonidet P40, 0.5% (v/v); Tween 20, 0.5% (v/v);<br>glycerol, 50% (v/v); pH approx. 7.5 at 20°C |
| Volume activity | ≥ 5 x $10^3$ U/ml |
| Endonucleases<br>(λDNA)<br>(MWM II)<br>Nicking activity (pBR322) | <br>not detectable (≤ 30 U/4 h/72°C)<br>not detectable (≤ 30 U/4 h/72°C)<br>not detectable (≤ 30 U/4 h/72°C) |
| Function testing in PCR<br>(λDNA, 0.5 kb)<br>(human genomic DNA, 1.1 kb) | <br>corresponds<br>corresponds |
| Stability | at –20°C 18 months |

**Description**

Thermostable Pwo DNA Polymerase was originally isolated from the hyperthermophilic archaebacterium Pyrococcus woesei. It is now supplied as a recombinant enzyme from E. coli. Pwo DNA Polymerase has a molecular weight of about 90 kD. It is a 5' – 3' DNA polymerase and has a 3' – 5' exonuclease (proofreading) activity. The enzyme has no detectable 5' – 3' exonuclease activity.



## Application

Because of its proofreading activity, Pwo DNA Polymerase amplifies DNA with tenfold greater accuracy than other common thermostable polymerases. It is especially useful for such demanding applications as

- Routine high fidelity amplification of up to 3 kb human genomic DNA targets.
- Amplification of genomic products for cloning.
- Study of allelic polymorphisms.
- Characterization of rare mutations.
- Characterization of cell populations.
- Labeling of PCR products with modified nucleotides (DIG-dUTP, biotin-dUTP, fluorescein-dUTP).

Pwo DNA Polymerase cannot amplify targets longer than approx. 3 kb. For high fidelity amplification of longer templates, see the product profiles of the Expand PCR Systems.

## Key advantages

- Maximum PCR fidelity, because the proofreading activity of Pwo DNA Polymerase excises misincorporated nucleotides.
- High PCR yield, because the polymerase is very resistant to the high denaturing temperatures used in PCR.
- Simplifies cloning of PCR products because it generates blunt-ended products.

## Properties

| | |
|---|---|
| Thermal stability | Pwo DNA Polymerase is stable for >2 h at 100°C. |
| Divalent ion requirement | $Mg^{2+}$ (standard concentration, 2 mmol/l).<br>The polymerase prefers $MgSO_4$ to $MgCl_2$. The enzyme is particularly sensitive to $Mg^{2+}$ concentration when the target is >2 kb long. |
| dNTP concentration | approx. 200 µmol/l for each dNTP. Caution: Lower deoxynucleotide concentrations can activate the 3' – 5' exonuclease proofreading activity, which might degrade primers and products. |
| Effect of additives | Usually, additives will not improve the performance of Pwo DNA Polymerase in PCR. Nevertheless, additions of up to 100 µg/µl bovine serum albumin (BSA), Triton X-100 (0.1%), pyrophosphatase, DMSO, or betaine may improve efficiency in some cases. |